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RAT CHOROID PLEXUS BLOOD VESSELS: MORPHOMETRIC CHARACTERISTICS

KRVNI SUDOVI HOROIDNOG PLEKSUSA PACOVA: MORFOMETRIJSKE KARAKTERISTIKE

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Ključne reči
Horoidni pleksus pacova, krvni sudovi, morfometrijska i ultrastrukturna istraživanja, razvoj.

Abstract

The choroid plexus consists of epithelial cells, fenestrated blood vessels, and the stroma, dependent on various physiological or pathological conditions.
In the present study morphometrical investigations of the rat choroid blood vessels during development (1, 1.5, 2, 4, 7, 10, 13 and 22 months) were carried out. The vessels number and luminal diameter of the blood vessels divided in four subgroups were measured. Morphometric investigations were performed on semithin toluidine blue stained sections examined with the light microscope using a combined square grid system calibrated for stereological estimations.
The statistical significant changes were observed in the luminal diameter of capillaries in the young rats in comparison to adult rats. Blood vessels with luminal diameter >30 μm have been found for the first time in rats aged 2 months. The capillaries number was reduced by 20% during development.
Changes of the rat choroid plexus during development, characterized by reduction of the capillaries and atrophy of epithelial cells, are evidence of initial degeneration and could result in decreased choroid plexus secretory and transport functions.

INTRODUCTION

Plexus choroideus is intraventricular brain structure involved in the production of cerebrospinal fluid (CSF) and in the synthesis and transport of numerous CSF components. The surface of the choroid plexus consists of numerous villi each covered with single layer of epithelial cells surrounded by vascular connective tissue cells [1, 2, 3]. These cells are generally considered to be modified ependymal cells with epithelial cell characteristics and referred to as choroidal light and dark epithelial cells (average size 13 μm) [4]. As a secretory source of vitamins, peptides and hormones for neurons, the choroid plexus provides substances for brain homeostasis [5].

Most blood vessels in plexus choroideus are wide-calibers (approximately 15 μm) capillaries with thin fenestrated endothelial walls and bridging diaphragms overlying the fenestrations [6].

Purpose of the present study is an investigation of morphometrical changes of the rat choroid plexus blood vessels during development from 1 to 22 months.

MATERIAL AND METHODS

Wistar rats (n=40) aged 1, 1.5, 2, 4, 7, 10, 13 and 22 months were used. The animals were fixed by immersion [7] and by intracardial perfusion [8]. The choroid plexuses were embedded in Durcupan and examined with JEOL JEM 1200EX transmission electron microscope. The semithin sections (1 μm) were stained with 1% toluidine blue for morphometric measurements and examined under light microscope.

Morphometric analysis

We obtained morphometric data from the light microscope Carl Zeis Jena at 1000x magnification using a square grid system (625 test point) [9] calibrated for linear measurement in μm and stereological measurement in μm². We measured the relative number of blood vessels and luminal diameter of the blood vessels divided in four subgroups. The luminal diameter was measured as perpendicular distance across the maximum chord axis of each vessel.

Statistical analysis

Results are reported as mean values ± SEM and as relative part in percentage, and statistically analyzed by Student's t-test using statistical package (STATISTICA, ver.6, Stat-Soft Inc., 2001).

The animal experiments were performed in accordance with animal protection guidelines approved by the Ethics Committee for experimental animal use at IEMPAM – BAS.

RESULTS

In the present study on the rat choroid plexus during development was measured relative number of blood vessels and cross-sectional diameter of the blood vessels divided in four subgroups. The changes in luminal diameter and number of the blood vessels are shown in Figures 1 and 2. There

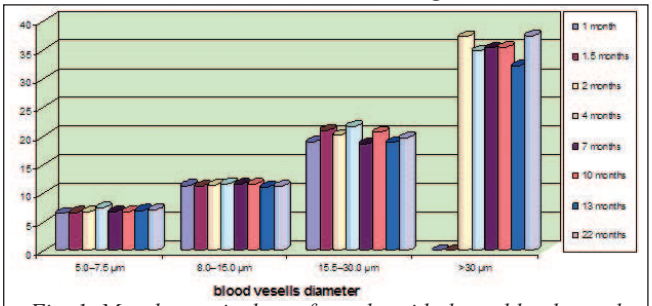

Fig. 1. Morphometric data of rat choroid plexus blood vessels during development (luminal diameter in µm)

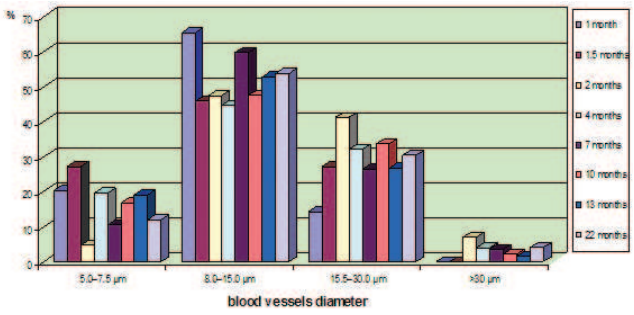

Fig. 2. Morphometric data of rat choroid plexus blood vessels during development (relative part in %)

was a little extension in the blood vessels diameter of the 5.0-7.5 µm of the 1 (6.54±0.29 µm) and 1.5 (6.72 ±0.21µm) months rats during development in comparison to 13 (7.06±0.22 µm) and 22 (7.18±0.25 µm) months rats ($p< 0.001$; $p<0.01$). No significant changes were observed in the blood vessels diameter of the 8.0-15.0 µm and 15.5-30.0 µm respectively during the whole period of development. Blood vessels with luminal diameter >30 µm have not been found in young rats (1 and 1.5 months), and this may be related to a larger number of the blood vessels with small luminal diameter, i.e. capillaries (85.80% and 72.88%) and blood vessels of 15.5 – 30.0 µm in diameter (14.20% and 27.12%). Blood vessels with luminal diameter >30 µm have been found for the first time in rats aged 2 months (6.87%). The mean relative part of the blood vessels >30 µm was 3.15% in the 4, 7 and 10 months rats and respectively 2.77% in the 13 and 22 months rats. In 4, 7 and 10 months rats the relative part of the capillaries is mean 66.19% and in 13 and 22 months rats is 68.72%.

The ultrastructural investigations of the rat choroid plexus during development are shown in Figures 3 – 7. The apical surface of the choroid plexus consists of small villi each covered with a single layer of large cuboidal epithelial cells (mean size of light and dark cells - 13 µm), electron-dense epithelial cytoplasm with many mitochondria, well differentiated connective tissue elements and basal membrane, and capillary with many fenestrations (Fig. 3). At the apical ends the lateral faces of contiguous epithelial cells form a tight junction that limits the exchange between the blood and cerebrospinal fluid (Fig. 4). In our investigation on the rat choroid plexus we used lanthanum nitrate, as electron-dense marker, perfused into the carotid arteries. The injected marker into blood supply crosses the fenestrated capillaries of the choroid plexus to enter the perivascular connective tissue space. The marker is present in numerous vesicles, moves into the interior of the cells, but does not pass the tight junctions. Thus the apical tight junctions do

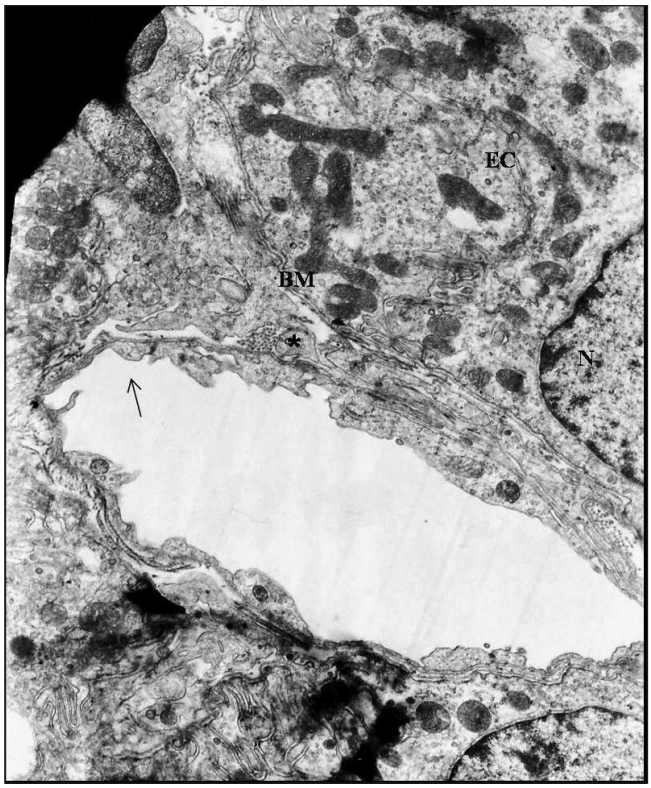

Fig. 3. Electron micrograph of rat choroid plexus aged 1 month; epithelial cell (EC) and nucleus (N); basolateral epithelial membrane (BM), connective tissue stroma (*) and fenestrated capillary (→). x 7 300

not permit the marker to enter into the cerebrospinal fluid (blood-cerebrospinal fluid barrier). The microvilli of the epithelial cells of the mature rat choroid plexus (4, 7 and 10 months) are short, with dilated endings and rarely arranged; the nuclei of the epithelial cells are rounded, located basally and have relatively homogenous chromatin (Fig. 5). Differences in the cytoplasmic electron densities between light and dark epithelial cells are more marked in rat aged 13 and 22 months. In the epithelial cytoplasm are seen many vacuoles, dense bodies, lipid droplets, imbibing mitochondria and second lysosomes (Fig. 6, 7). The connective tissue space is dilated (Fig. 7).

DISCUSSION

Although the great majority of the capillaries within the brain are of the continuous type and maintain the blood-brain barrier (BBB), the small specialized regions that make up the circumventricular organs (CVOs) lie outside the BBB. The CVOs include the choroid plexus, pineal gland, median eminence, subfornical organ, vascular organ of the lamina terminalis and area postrema. The blood vessels in these regions differ from those in the rest of the brain in that they contain fenestrated and continuous muscle-type capillaries [10]. Up to present time little quantitative information has been available regarding the vessels of the brain and, in particular, those of the rat choroid plexus during development. The present morphometrical study of the rat choroid plexus showed that the diameter of capillaries (vessels <15.0 μm in diameter) and that of large vessels (vessels of 15.5 – 30.0 μm and >30.0 μm in diameter) did not change during development. There was only a little extension in the blood vessels diameter of the 5.0 - 7.5 μm of the young rats (1 month – 7.95% and 9.79%; 1.5 months – 5.06% and 6.85%) in comparison to adult rats (13 and 22 months). The mean luminal diameter of capillaries (blood vessels diameter of 5.0 – 15.0 μm) was 9.01±0.43 μm in the young (1, 1.5 and 2 months), 9.30±0.39 μm in the mature (4, 7 and 10 months) and 9.17±0.27 μm in the adult (13 and 22 months) rats.

In previous our study the blood vessels with luminal diameter >30 μm have not been found in young rats (1 month) and this may be related with a large number of the blood vessels with small luminal diameter, i.e. capillaries [11]. In the present study the blood vessels with luminal diameter >30 μm were found for the first time in rat aged 2

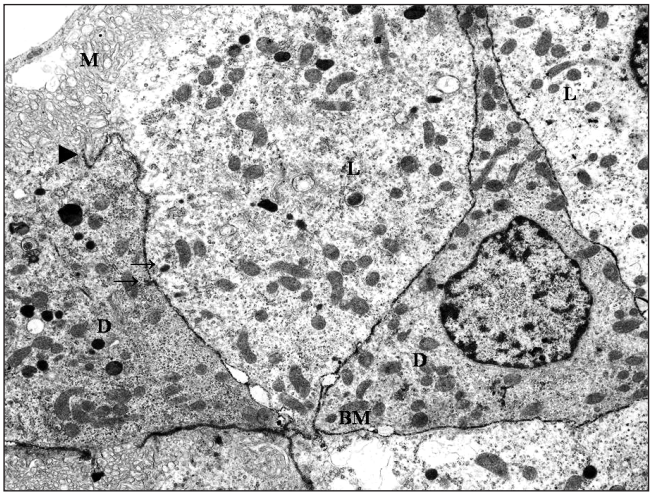

***Fig. 4**. Electron micrograph of rat choroid plexus aged 4 months; microvilli (M); light (L) and dark (D) epithelial cell; basal epithelial membrane (BM); numerous pinocytotic vesicles, filled with lanthanum nitrate - electron-dense marker (→); apical tight junction (▶). x 4 000*

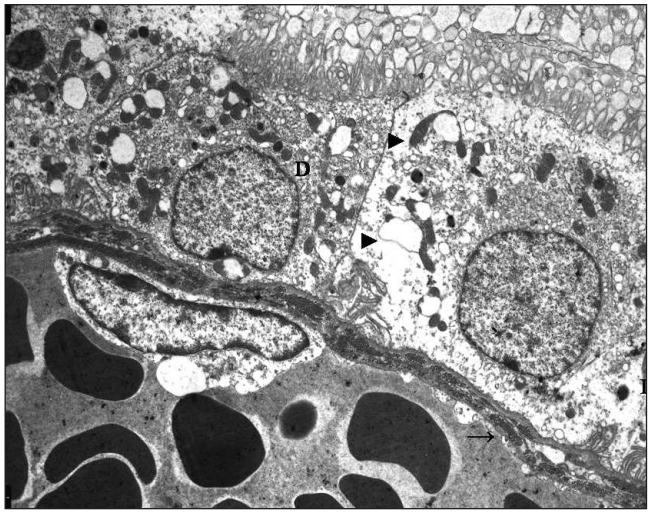

Fig. 6. Electron micrograph of rat choroid plexus aged 13 months; light (L) and dark (D) epithelial cell with imbibing mitochondria (→) and dense bodies; blood vessels (▶). x 3 000

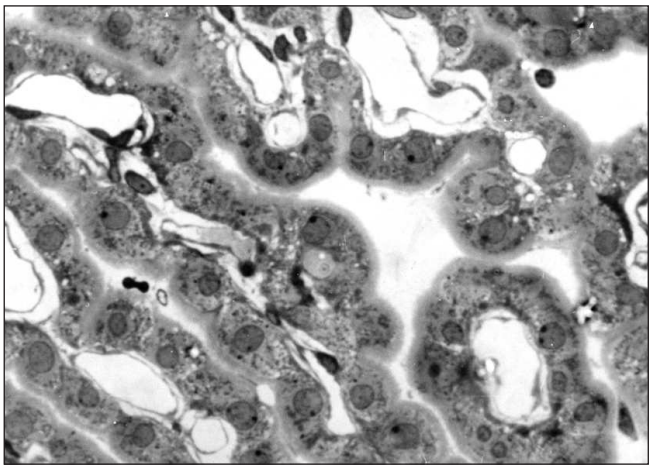

***Fig. 5**. Photomicrograph of semithin section of rat choroid plexus aged 7 months; many cubic light and dark epithelial cells with round nucleus, thin apical microvilli and capillaries; Hematoxylin and eosin stain. x 2 872*

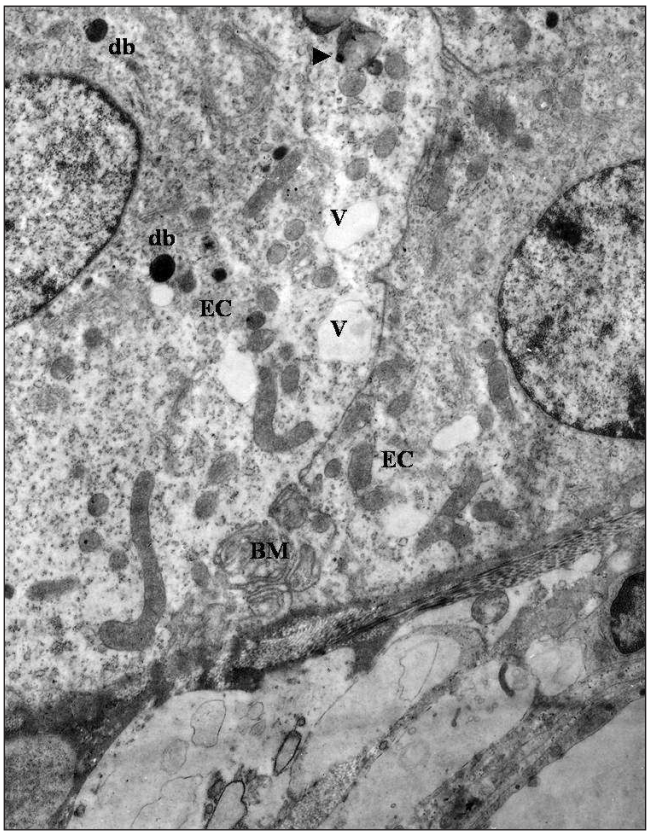

Fig. 7. Electron micrograph of rat choroid plexus aged 22 months; epithelial cells (EC) with many vacuoles (V), second lysosomes (▶) and dense bodies (db); basolateral membrane (BM); connective tissue stroma (*); fenestrated capillary (→). x 5 000

months. The capillaries number (blood vessels with diameter 5.0 – 15.0 μm) was reduced by 20% during development (1 month – 85.80%; 22 months – 65.70%) and this compensatory reaction may be related with age-related changes.

The ultrastructural changes of the rat choroid plexus with age (lipid droplets, lysosomes, dense bodies) might indicate a gradual change in function or at least a decrease in efficiency of the choroid plexus. These changes are evidence of slow degeneration of the rat choroid plexus with age [11, 12]. The presence of imbibing mitochondria could lead to a decrease in both potential energy content and secretion of cerebrospinal fluid.

The two barriers that represent the largest interface between blood and brain extracellular fluids, the BBB and the blood-cerebrospinal fluid barrier (BCSFB), prevent the free paracellular diffusion of polar molecules by complex morphological features, including tight junctions that interconnect the endothelial (brain capillary endothelial cells) and epithelial cells (choroid plexus epithelial cells), respectively [13]. The changes in the choroid plexus indicate that normal aging processes alter protein content in the CSF, CSF secretion and integrity of the BCSFB, which could impact on CSF homeostasis and turnover [14, 15].

CONCLUSION

These results indicate that there is little extension of capillaries luminal diameters during ontogenesis. The increase of capillaries diameter in the adult rats represents a process directed to compensation of the reduction of the microvessels and reflects the developed age-related changes of the brain. In young rats (1 and 1.5 months) the blood vessels with luminal diameters >30 μm have not been found and this may be related with a larger number of the blood vessels with small luminal diameter, i.e. capillaries. Blood vessels with large luminal diameter for the first time have been found at the age of 2 months. The ageing of rat choroid plexus is characterized by reduction of capillaries and atrophy of epithelial cells. These functional changes are evidence of choroid plexus slow degeneration.

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Apstrakt

Horoidni pleksus se sastoji od epitelnih ćelija, fenestriranih krvnih sudova i strome, u zavisnosti od različitih fizioloških ili patoloških stanja.

U ovom istraživanju urađena su morfometrijska ispitivanja krvnih sudova horoidnog pleksusa pacova u toku razvoja (1, 1,5, 2, 4, 7, 10, 13 i 22 meseci). Broj krvnih sudova i promer lumena krvnih sudova podeljeni su u četiri podgrupe i mereni. Morfometrijska istraživanja su urađena na polutankim isečcima obojenim toluidin plavo i ispitativana svetlosnim mikroskopom koristeći kombinovani sustem kvadratne mrežice kalibrisan za stereološku analizu.

Statistički značajne promene zabeležene su u promeru lumena kapilara u mladih pacova u odnosu na odrasle. Krvni sudovi lumena promera> 30 μm su pronađeni prvi put na pacovima u dobi od 2 meseca. Broj kapilara tokom razvoja. je smanjen za 20% .

Promene horoidnog pleksusa pacova tokom razvoja karakteriše smanjenje broja kapilara i atrofija epitelnih ćelija, a dokaz su o početnoj degeneraciji koja može dovesti do smanjenja sekretorne i transportne funkcije horoidnog pleksusa

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